

the conditions for expression of gene LP2-3. Thus, the system allows a rapid determination of stage specific embryos by a simple phenotypic reporter screen, perhaps by visualization of green fluorescent protein (GFP) or by loss of fluorescent protein product. Similarly, a set of promoters from known, differently staged genes operatively linked to reporter genes will be effective for monitoring developmental changes within the system as the embryos mature. The LP2-3 promoter is identified as SEQ ID NOS: 328-334 in Table I. The promoter expression pattern is that of the natively linked gene, LP2-3.

[0108] Virtually any indicator or reporter gene can be used for this approach or for other methods associated with this invention provided they are compatible with the system studied. Generally, reporter genes are genes typically not present in the recipient organism or tissue and which encode for proteins resulting in some phenotypic change or enzymatic property. Examples of such genes and assays are provided by Schenborn, E. and Groskreutz, D., Mol. Biotechnol., 13:29, 1999; Helfand, S. L. and Rogina, B., Results Probl. Cell Differ., 29:67, 2000; Kricka, L.J., Methods Enzymol., 305:333, 2000; Himes, S.R. and Shannon, M. F., Methods Mol. Biol., 130:165, 2000; and Leffel, S. M. et al., Biotechniques, 23:912, 1997, which are incorporated in their entirety by reference. In one embodiment of this invention, the reporter used is GFP, or any ariant of the fluorescent protein.

[0109] Additionally, one skilled in the art would recognize that a promoter, like that from LP2-3, has potential to stimulate production of products not ordinarily observed at a particular stage. A promoter derived from a gene that expresses during a known stage, for example an early stage, can be operatively linked to a gene that does

not normally express during that stage, yielding controlled expression of any targeted gene. It may be shown that earlier or later expression, or prolonged expression of a particular gene may give a desirable genotype or phenotype in a mature plant, may result in increased vigor in culture, or may be sufficient to alter the normal maturation process of the embryo. Prolonged expression of any desired gene also may be achieved from linking a constitutively expressed promoter to the targeted gene. Further, the ability to manipulate gene expression during embryogenesis allows for a detailed study of the effects of an individual gene or multiple genes on embryogenesis, leading to a better understanding of the developmental processes involved in embryogenesis.

Method of Correlating Gene Expression with Improved Tree Stock or Culture Conditions

[0110] Importantly, the cDNAs and related molecules of the invention can also be used as markers to examine genetic heterogeneity and heredity through the use of techniques such as genetic fingerprinting. These markers can also be correlated with improved agronomic traits including good initiation frequency, embryonic maturation, high frequency of germination, rapid growth rates, herbicide tolerance, insect resistance, pathogen resistance, climate and environmental adaptability wood quality, and wood fiber quality and content, to name a few. Additionally, the expression of these developmentally regulated genes can be compared among genetically identical clones grown under different culture conditions to determine the best protocols and media for somatic embryogenesis.

[0111] Cryogenic storage of pine tree embryos is effective for maintaining stocks of embryos determined by this invention to have the desired fitness traits or exist at the

LAW OFFICES

FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

appropriate developmental stage. With such storage, one can specifically target desirable embryos for expansion many years after they are frozen. For example, a culture of somatic embryos can be divided into at least three portions, one of which is cryogenically stored, one which is used to study gene embryonic gene (and protein) expression, and one that is used to produce seedlings for field testing. Clones producing valuable mature plants could be selected and expanded from frozen stocks. Additional clones exhibiting similar expression patterns could be selected for future expansion and cultivation.

[0112] As will be evident to the ordinary practitioner, there are numerous ways in which the nucleic acids, polypeptides and antibodies of this invention might be used to characterize the gene expression of embryos. Ideally the stage-specific gene expression of embryos of several different genotypes and at several different stages of embryogenesis are characterized. For example, sets of oligonucleotide primers designed using any one of SEQ ID NOS: 1-327 may be used in RT-PCR assays to characterize expression of a gene product. *In situ* hybridization assays or antibody staining protocols may also be used to characterize RNA and/or protein expression and localization.

[0113] Embryos of the same genotype in which gene expression has been characterized may also be used to generate plantlets that are used in field testing. Once the embryos have developed into mature trees, the various genotype trees can be evaluated for important traits such as growth rates, herbicide tolerance, insect resistance, pathogen resistance, climate and environmental adaptability, wood quality, and wood fiber quality and content, among others. Finally the phenotypic data collected

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FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000